Influence of the time of capture on Lipid content of the Meagre Produced in Aquaculture

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Food Engineering

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ACRONYMS AND ABBREVIATIONS

AA - Arachidonic Acid

ALA - Alpha-Linolenic Acid

CH - Cholesterol

DHA - Docosahexaenoic Acid

EFA - Essential Fatty Acids

EPA - Eicosapentaenoic Acid

FAME - Fatty Acid Methyl Esters

FAO - Food Agriculture Organization

FFA - Free Fatty Acids

GC - FID - Gas Chromatography and Flame Ionization Detector

IUPAC - International Union of Pure and Applied Chemistry

LA - Linoleic Acid

LDL - Low Density Lipoprotein

LWE - Live Weight Equivalent

MUFA - Monounsaturated Fatty Acids

PCA - Principal Component Analysis

PL - Phospholipids

PUFA - Polyunsaturated Fatty Acids

SFA - Saturated Fatty Acids

TAG - Triacylglycerol

TL - Total Lipids

TLC - Thin Layer Chromatography

RESUMO

Ultimamente os consumidores têm estado cada vez mais preocupados com a saúde e o seu bem-estar e, consequentemente, com a sua dieta. A tendência de consumo dos produtos da pesca aumentou pelo que a aquacultura se tornou cada vez mais importante.

A Corvina (*Argyrosomus regius*) é um dos maiores *Sciaenídeos*. A corvina é um peixe com excelentes características biológicas para a aquacultura, tolerante a uma ampla gama de salinidade e com elevado valor comercial. A qualidade do músculo é considerada muito boa, caracterizada por um elevado teor de proteínas e baixo teor de lípidos.

O presente trabalho teve como objetivo o estudo do impacto da época do abate na qualidade da corvina, nomeadamente da frações lipídicas (o teor de lipídios totais, classe de lipídios, perfil dos ácidos gordos). Foram analisados amostras do mês de Abril e de Novembro. A análise evidenciou que a corvina é um peixe magro. A composição química da corvina apresenta alguma sazonalidade sendo a fração de lípidos a que mais varia ao longo do ano. Verificou-se que os valores do teor lipídico (8.6 %), o EPA (3.3 %), a razão ómega3/ómega6 (1.1 %) foram significativamente maiores no Outono. Em Abril mais rico em ácidos gordos ómega-3, destacando-se o DHA (10.3%) e ómega-6, em particular LA (19.3%). Conclui-se assim que o músculo da corvina no Outono evidencia a correlação com o alimento composto.

Palavras-chaves: *Argyrosomus regius*; musculo; teor de lipídios totais; ω3/ω6.

ABSTRACT

Lately the consumers have been more concerned with their health and well-being, and consequently, with their diet. Trends for the consumption of fishery products had increased, and as a result, aquaculture has become increasingly more important in this context.

The meagre (*Argyrosomus regius*) is one of the largest *Sciaenidae*. The meagre is a fish with excellent biological characteristics for aquaculture, such as tolerance to a wide range salinity and high commercial value. The muscle quality is considered very good, with high protein content and low fat content.

The present work has as a goal to study the impact of the season of capture in the quality of the meagre, namely the lipid fractions (the total lipids content, the lipids classes, and the fatty acids profile). Samples from November and April were analyzed. This analysis found that the meagre is a low fat fish. The chemical composition of the meagre presents some seasonality, the lipids fractions being the composition that varies the most along the year. The values of lipid content (8.6%), EPA (3.3%), and omega3/omega6 (1.1%) were significantly higher in autumn. In April the samples had more omega3 fatty acids, like the DHA (10.3%), and the omega-6, in particular LA (19.3%). It is therefore concluded that the meagre muscle in the autumn has a correlation with the feed.

Keywords: *Argyrosomus regius*; muscle; total lipid content; ω3/ω6.

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1. INTRODUCTION

1.1. Aquaculture and its importance

Today aquaculture production is developing rapidly all over the world. Despite the growing demand for aquatic products and lack of good production in meat products, aquaculture has already made the cultivation more attractive. For the Food Agriculture Organization (FAO), aquaculture has been identified as the fastest growing food sector in the world. As the global population is increasing so the demand for fresh fish is also growing. Aquaculture is regarded as an effective method to overcome the scarcity of protein, especially because consumer habits are changing into more healthy diets, and thus interest by the public in fish products is increasing (NOAA, 2011).

According to FAO (2014), Aquaculture can be defined as the production technique of aquatic life from fish, mollusks, crustaceans and aquatic plants. This term includes the cultivation of both marine and freshwater species. Its goal is to increase production through practices such as artificial feeding, protection from predators and integration with other species. Thus, aquaculture presents several advantages, including sustainable fishing, higher yields due to freshwater paths, and the largest quantity of fish compared to wild production. All this advantages help global food production, the conservation of species, and give economic support especially for marine coastal aquaculture.

According to FAO (1999), the demand for fish increased by about 31% between 1990 and 1997, while fishing just recorded an increase of 9% the same period. World fish consumption per capita has increased and is estimated to have reached an average of 16.5 kg in 2003 (FAO, 2006). World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (IOI, 2014).

1.2. The World's Aquaculture Situation

1.2.1. Overview

In the past twenty years there has been a significant increase in aquaculture production worldwide with Asia continuing to dominate the production. China is the country that has the highest share, making up to 85.3% of Asian production and 77.6% of global production (FAO, 2001). Global aquaculture production in 1999 was 42.8 million tons. Chinese aquaculture production in 2000 was 25 million tons, consisting of freshwater culture (FAO, 2002a).

Aquaculture's contribution increased from 638 577 tons in 1950 to 54 785 841 tons in 2003. The sector has grown 8.8 % *per* year since 1950, compared with the increase of 3.0 % *per* year for total capture fisheries landings. In 2006, global aquaculture production was estimated at 85.9 million tons and global industrial feed output exceeded 635 million tons (FAO, 2008).

In the Mediterranean area total aquaculture production has increased. Production of marine fish species has grown while production of mollusks has decreased. Egypt, France, Spain, Italy, Turkey and Greece are the main producing countries (Paquotte *et al.,* 2008).

1.2.2. European Market for Seafood and Aquaculture Products

The European Union (EU) is the world's largest fish market and has become increasingly dependent on imports to meet its needs. In 2007, The EU imported about 23 billion euros in fish and fisheries products from non-EU suppliers (Monfort, 2010). The biggest importers are Spain with 4.7 billion euros, France with 3.9 billion euros, and Italy with 3.7 billion euros. The export of Europe in 2008 was around 2.7 billion euros (Zampogna, 2009).

Between countries there are obvious changes in the type of consumed products. In southern European countries there is a wide range of supply of fish species, while in most northern countries this offer is already more limited. In Mediterranean countries, the whole fish of purchase is still a common practice, whereas in northern European markets the fish is processed and packaged in individual doses before reaching the final consumer (Monfort, 2010).

The fish consumption in the EU was about 13.3 million tons in 2007, of which 10.5 million tons are from fish caught and 2.8 million tons are from aquaculture (Monfort, 2010). The market position of aquaculture products for fishery products is still low, but is growing.

1.2.3. Aquaculture in Portugal

Portugal has strong tradition in the fishing sector since long ago because of its long mainland coastline. The fishing industry is of particular importance to Portugal and mostly as a major means of subsistence. Portugal presents the highest fish consumption *per capita* in Europe. The average annual consumption of seafood by the Portuguese population is estimated at about 61.10 kg *per capita* in 2009 and making Portugal one of the four countries in the world with a diet based on this type of food (Vilhena Sykes, 2010).

kg/capita/year	2010	2015	2020	2025	2030
Portugal	59	59	58	58	57
Spain	39	39	39	39	39
France	32	32	32	33	33
Italy	25	26	27	28	29
Greece	26	26	27	27	27
Belgium-Luxembourg	22	23	23	23	24
Europe	22	23	23	24	24

Figure 1 – Potential of the aquaculture sector in Portugal. Fonte: FAO Fisheries Circular No. 972/4, Part 1.

According to the Portuguese Association of Aquaculture (2014), the fish consumption in Portugal should continue to be one of the highest in the world (Figure 1). Consumption in Europe will remain stable in some countries and increase in others.

In 2012, Portugal imported more than 420.000 million tons fish and fishery products for a total worth of 1.8 billion euros and exported 1.0 billion of fish and sea food products worldwide, a decrease of 8% from 2011 (International Markets Bureau). Cod is by far the first imported species, either deep-frozen or salted (Paquotte *et al.,* 2008).

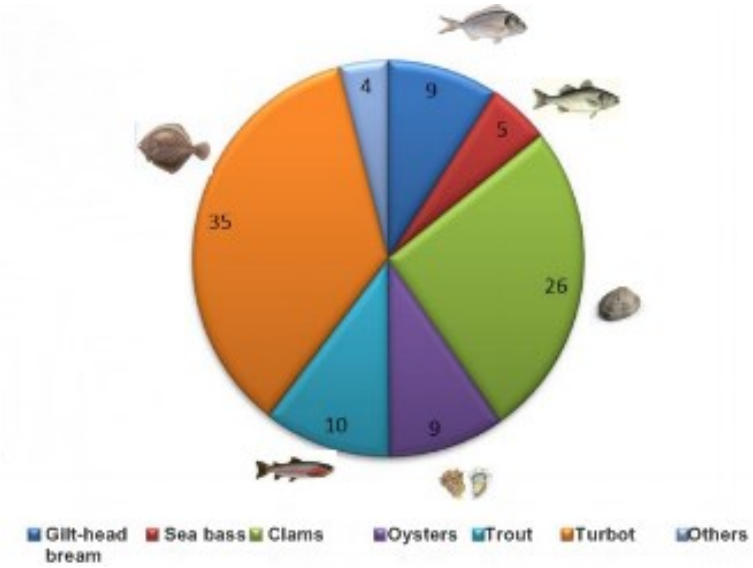


Figure 2 – Main species produced in Portugal

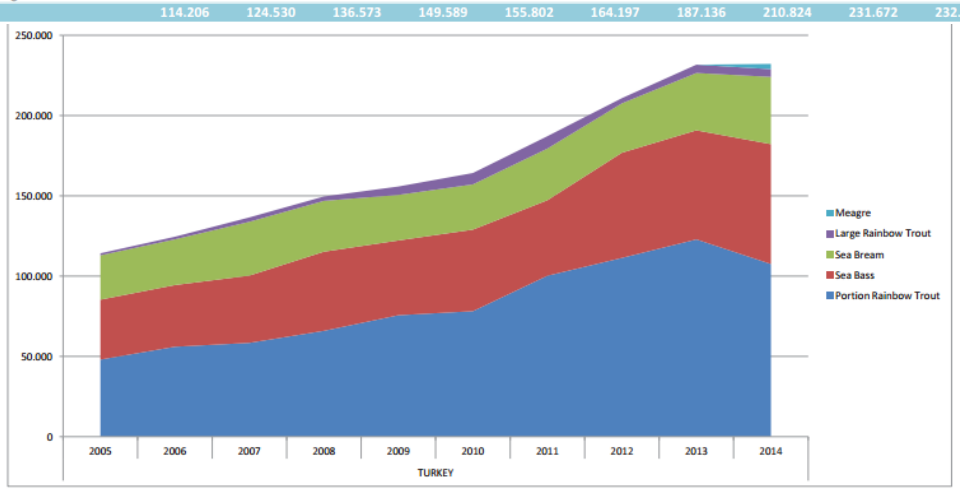
1.2.4. Aquaculture in Turkey

Turkey is located at the junction of two continents, Asia and Europe, surrounded by seas on three sides, the Black Sea in the north, the Mediterranean in the south and the Aegean in the west. Aquaculture in Turkey started with rainbow trout (*Oncorhynchusmykiss*) culture in 1970. In 1985 the first sea bream (*Sparusauratus*) and sea bass (*Dicentrarchuslabrax*) hatchery was established by Pınar Sea Products and production started in the Aegean Sea. The first official aquaculture statistics were published in the following year and marine fish culture was about 35 tons. As a result Turkey is becoming an important player in world aquaculture. Latest developments in the aquaculture sector place Turkey in an important position both in the Mediterranean basin and among the EU countries (Gozgozoglu *et al.,* 2010; Candan, 2007). Turkish aquaculture production reached 188 790 tons in 2011. The three biggest species are rainbow trout (53% of total aquaculture production), sea bass (25%) and sea bream (17%). There is also production of sea trout (4%) and new Mediterranean species (1%) (Aydin et al., 2014).

Current *per capita* fish consumption in Turkey is very low comparing to many European countries, but it is expected that the recent developments will lead to increases in domestic fish consumption. In each region in Turkey there are different levels of fish consumption. For example, *per capita* consumption is 25 kg in Black Sea coasts, 16 kg in metropolises (Ankara, Istanbul, Izmir) and 0.5 kg in East and South East Anatolia regions (Knudsen, 2009).

Figure 3 – Turkey production tons (no official data available before 2014) Adapted from FEAP, 2015.

TURKEY	Portion Rainbow Trout	48.033	56.026	58.433	65.928	75.657	78.165	100.239	111.335	122.873	107.533
	Sea Bass	37.290	38.408	41.900	49.270	46.554	50.796	47.013	65.512	67.912	74.653
	Sea Bream	27.634	28.463	33.500	31.670	28.362	28.157	32.187	30.743	35.701	41.873
	Large Rainbow Trout	1.249	1.633	2.740	2.721	5.229	7.079	7.697	3.234	5.186	4.812
	Meagre*										3.281
TURKEY Total		114.206	124.530	136.573	149.589	155.802	164.197	187.136	210.824	231.672	232.152



1.3. Meagre (*Argyrosomusregius*)

Argyrosomusregius is a good candidate species for diversification of Mediterranean aquaculture due to the fast growth of juveniles in sea cages. Also known as meagre, shade-fish, salmon basse or stone basse, it belongs to the family *Sciaenidae*. *Argyrosomusregius* is found in the Mediterranean and Black Sea and along the eastern Atlantic coast (Figure 4). It has a similar shape to a European seabass, with pearly-silver coloration and a yellow colored mouth, with relatively big head, the mouth in terminal position and an elongated body. Its length can reach up to 2m and it weights up to 55 kg. It is called meagre in England, maigre in France, corvina in Spain, corvina in Portugal, granyöz or kaya levreği in Turkey and mylokope in Greece.

Figure 4 – Argyrosomusregius (left). Geographical distribution of Meagre (right).

High meat quality, high commercial value, fast growth between 16 and 20°C, wide range of salinity tolerance, excellent biological characteristics for captivity, and also high nutrient content can be counted as some of the advantages in the culture of meagre (Tocher, 2003). The species inhabits in inshore and shelf waters close to the seabed. They congregate inshore to spawn during spring and summer in estuaries and females can lay up to 800 000 eggs (Zampogna, 2009).

The production of meagre began in the second half of the 90s, following an agreement between Italian and French producers, which resulted in the first commercial production of meagre in 1997 in France. Today, meagre is produced in several countries in the Mediterranean basin and its production increased rapidly (Table 1).

Table 1 – Meagre production in tons. Adapted from FEAP, 2015.

PRODUCTION (tons)		YEAR									
SPECIES	COUNTRY	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
	CYPRUS	0	0	0	0	0	12	30	30	48	33
	FRANCE	267	282	235	206	121	268	500	420	200	377
	ITALY	320	280	335	300	320	320	300	300	190	190
	PORTUGAL	47	23	27	15	44	38	0	0	0	0
	SPAIN	273	385	810	1.300	1.660	3.250	2.880	1.640	90	1.090
	TURKEY*										3.281
Meagre Total		907	970	1.407	1.821	2.165	3.908	3.750	2.430	560	5.021

*no official data available before 2014

The quality of meagre meat is considered very good, characterized by a high protein and low lipid content. The lipids in meagre meat have a high content of polyunsaturated fatty acids (PUFAs), mainly represented by a high proportion of ω3 PUFA and low relation between the ω3/ω6 values (Monfort, 2010).

Table 2 – Profile of fatty acids, mineral and vitamin content and chemical composition of meagre. Adapted from Bandarra *et al.,* 2004.

Fatty acids (mg/100g)-Crude		Minerals (mg/100g)-Crude		Chemical Composition(g/100g) – Crude		Vitamins(/100g)-Crude	
16:0	234,7	**Ca**	13,0	**Moisture**	76,7	**A(µg)**	1,3
SFA	342,3	**P**	233	**Fat**	1,4	**E(mg)**	0,5
18:1 ω9	144,2	**Mg**	31	**Ash**	1,2	**D(µg)**	16,0
MUFA	465,0	**Fe**	0,3	**Protein**	20,4	**B1(mg)**	0,1
18:2 ω6	8,0	**Na**	56			**B2(mg)**	0,1
EPA	5,7	**K**	430			**B6(mg)**	0,3
DHA	147,0	**Mn**	<0,02	This fish is a good source of Potassium		**B12(µg)**	n.a
PUFA	233,8	**Cu**	<0,03			**Folates(µg)**	12,0
ω3	216,1	**Zn**	0,5				
ω6	17,7						

1.3.1. Chemical composition and nutritional value of fishery products

The fishery products are key foods in a balanced diet, presenting a high nutritional value, providing the beneficial effects to health and development of the human organism. Knowledge of the chemical composition of fishery products is one of the key aspects to assess their nutritional value, as well as the benefits associated with its consumption.

As with any product, the major components of fishery products are water, proteins and lipids. Fisheries products are also made up of minor components, such as minerals and vitamins. Table 3 shows the chemical composition of some species of fish.

Table 3 – Chemical composition of some fish products. Adapted from Bandarra *et al.,* 2004.

	Chemical Composition			
Species	**Water (%)**	**Protein (%)**	**Fat (%)**	**Ash (%)**
Cod	76.2	19.0	0.4	3.4
Monkfish	80.4	17.9	0.2	1.1
Mackerel	64.3	20.3	13.4	1.4
Tuna	69.7	24.8	3.5	1.7
Salmon	60.5	21.9	16.2	1.3
Octopus	83.1	15.9	1.2	0.9
Cockle	76.0	14.7	3.3	2.3
Crayfish	76.1	20.9	0.5	2.1

Currently it is considered that the importance of fishery products is clear from the major compounds, in particular lipids. These compounds are described below, with particular attention to lipids.

Water

The main component of fish muscle is water and varies in inverse ratio of the lipid content. The lean species have moisture content higher than the fatty species. This variation is more pronounced at the time of spawning season, in which occurs a reduction in energy reserves of fish (Ghaly et al., 2013).

Proteins

Proteins are a major constituent of fishery products. They are organic molecules that contain carbon, hydrogen, oxygen, nitrogen and frequently sulfur (Jobling, 1995). Most fish protein contents are between 16% and 21% whereas shellfish have slightly lower values (Bandarra *et al.,* 2004). Proteins derived from fish are nutritionally superior when compared to those of plant sources. They have a better balance of the dietary essential amino acids compared to all other animal protein sources (Ghaly *et al.,* 2013). Table 4 shows the estimated protein requirements of juvenile fish of a variety of species, and for meagre it is estimated at about 47% (Suloma, 2012).

Table 4 – Estimated protein requirements of some juvenile finfish. Adapted from Wilson, 2002.

Species	Protein Source	Estimated requirement (%)	Reference
Asian sea bass *(Lates calcarifer)*	Casein, gelatin	45	Boonyaratplin (1991)
Atlantic Salmon (*Salmo salar*)	Fish meal	55	Grisdale-Helland and Helland (1997)
European sea bass (*Dicentrarchus labrax)*	Fish meal	50	Hidalgo and Alliot (1988)
Florida pompano (*Trachinotus carolinus*)	Fish meal, soy meal	45	Lazo *et al*., 1998
Gilthead seabream (*Sparus aurata*)	Casein, FPC, amino acids	40	Sabaut and Luquet (1973)
Largemouth bass (*Micropterus salmoides*)	Casein	40	Anderson *et al*., 1981*
Meagre *(Argyrosomus regius)*	Fish meal	47	Martinez *et al*., 2011
Mulloway (*Argyrosomus japonicus*)	Fish meal	44- 49	Pirozzi *et al*., 2010
Rainbow trout (*Oncorhynchus mykiss*)	Casein, gelatin	40	Zeitoun *et al*., 1973*
Red drum *(Sciaenops ocellatus)*	Fish meal, casein	35- 45	Daniels and Robinson (1986); McGoogan and Gatlin (1998)
Red sea bream (*Pagrus major*)	Casein	55	Yone (1976)*
Yellowtail (*Seriola quinqueradiata*)	Fish meal	55	Takeda *et al*., 1975*

Lipids

Lipids are an important component of diet, both as energy and essential fatty acids (EFA) sources, which are needed in basic biological functions, like growth, reproduction and healthy issues (Huss, 1995). Lipids are not soluble in water but they are soluble in organic solvents (ether, acetone, mixture of chloroform-alcohol). Lipids can be divided into two groups: polar lipids mainly composed of phospholipids that contribute in the cell membrane formation are also called structural lipids and non-polar lipids, mainly consisting of triacylglycerol (TAG), free fatty acids, cholesterol and other sterols (Tocher, 2003).

Vitamins and Minerals

Sea foods are important sources of micronutrients such as vitamins and minerals. The amount of vitamins and minerals existing in fish is specific to each species. Vitamins are divided into two groups based on solubility, water soluble and fat soluble (Halver, 2002). For the water-soluble, the fish is very rich in B vitamins (B6 and B12) essential for the metabolism of carbohydrates and for the formation of red blood cells (NRC, 2011). In the class of fat-soluble highlights the vitamin A very important for vision and vitamin D plays a critical role in the absorption of calcium and phosphorus in the intestine. Also, the significant presence of vitamin

E in some species is indispensable to the body, since it acts as a natural antioxidant and reduces LDL content (Irish Sea Fisheries Board, 2008).

The fisheries and aquaculture products provide a wide variety of compounds minerals and are an excellent source of potassium, magnesium, calcium and phosphorus. According to Huss (1995), in the group of minerals also the salt water fish exhibits very high levels of iodine and selenium.

1.3.2. Importance of dietary lipids and essential fatty acids

Fatty acids are the simplest unit of lipids. They consist of a carboxyl group, located at the end of the molecule, which confers an acid character and a non-functional methyl group at the opposite end. The fatty acids are produced in the liver and subsequently transferred to other tissues (Horton et al., 1996).

However the terminology of fatty acids should obey a universal IUPAC nomenclature (International Union of Pure and Applied Chemistry). Thus, the fatty acids are shown based on: i) the length of its chain; ii) the degree of unsaturation (number of double bonds) present and iii) the position of the double bonds. Thus, in the usual representation the first number corresponds to the number of carbon atoms of the fatty acid; the second number corresponds to the number of double bonds present and the last number is the position of the carbon atom corresponding to the first double bond from the carboxyl group. In certain circumstances, it may become advantageous to indicate the position of the double bonds relative to the more distant edge of the carboxyl group, which is the methyl group whose carbon is termed "n" or "ω".

The uses of the Greek alphabet denote a mode of representation of the fatty acids that is becoming more popular. According to this representation, the carboxyl group carbon atom is the first but remains outside the score, causing the second chain carbon atom take α designation (alpha), the third the β designation (beta) and so on until the carbon methyl group receives the designation ω (omega). The position occupied by the double bonds is indicated with respect to the methyl group carbon, i.e., the ω carbon (Figure 5).

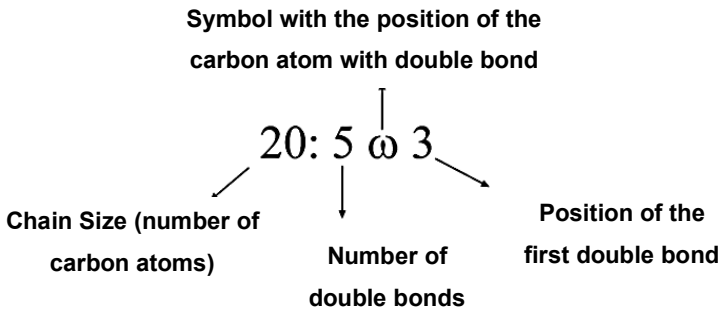


Figure 5 – International Nomenclature of fatty eicosapentaenoic acid with use of the Greek alphabet.

Dietary lipids are commonly classified as saturated, monounsaturated, or polyunsaturated. For each of these three classes exists a large number of different chemical variations or isomers. Saturated fatty acids (SFA) have a chain of carbon atoms joined by single bonds. The chain of carbon atoms is fully saturated with hydrogen atoms (Scrimgeour & Harwood, 2007). The SFA most common in fish are myristic (14:0), palmitic (16:0) and stearic (18:0). Diets high in saturated fat have been linked to chronic disease, specifically, coronary heart disease.

Monounsaturated fatty acids (MUFA) present a double bond in the fatty acid chain with all of the remainder carbon atoms being single bonded. The most common monounsaturated fatty acids are palmitoleic acid (16:1 ω7) and oleic acid (18:1 ω9) (Figure 6).

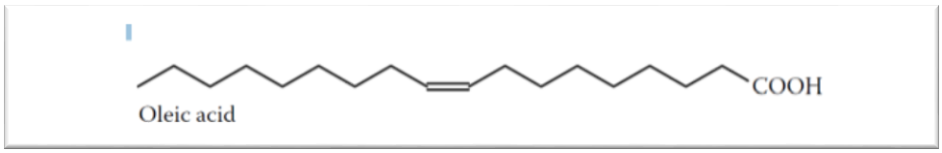


Figure 6 – Oleic acid representation. Adapted from Gunstone *et al.,* 2007.

The essential fatty acids (not synthesized) are considered all belonging to the group of PUFAs. When they are given sufficient amounts of ω3 and ω6 fatty acids (such as alpha-linolenic and linoleic) in the diet, some animals have the capacity to synthesize other fatty acids of the family ω3 and ω6 using desaturations and elongations (Parrish, 2009). Thus, essential fatty acids, linoleic (LA) and alpha-linolenic (ALA), respectively are the families of ω6 and ω3 precursors (Figure 7).

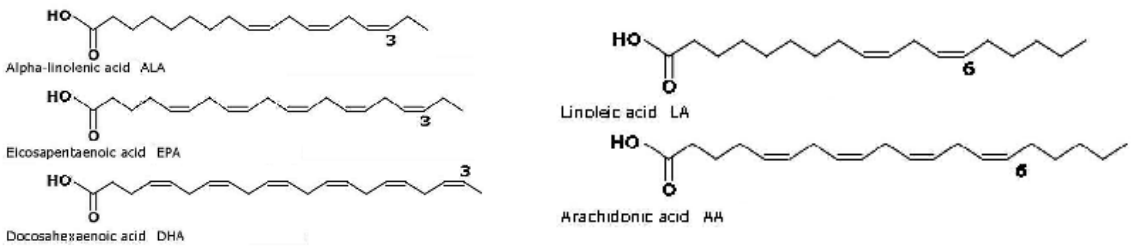


Figure 7 – Omega–3 (right) and Omega–6 (left) fatty acids.

The ω6 and ω3 are obtained from the diet or produced by the organism from LA and ALA by the action of elongases and desaturases enzymes. The elongases act adding two carbon atoms to the initial part of the chain, while the two desaturases oxidize chain carbons, yielding a double bond with cis configuration (Covington, 2004). The ω6 series is derived from the LA and ALA from ω3 series. From these essential fatty acids are then synthesized arachidonic acid (AA), eicosapentaenoic (EPA) and docosahexaenoic (DHA). The following figure illustrates the synthesis of fatty acids ω3 and ω6.

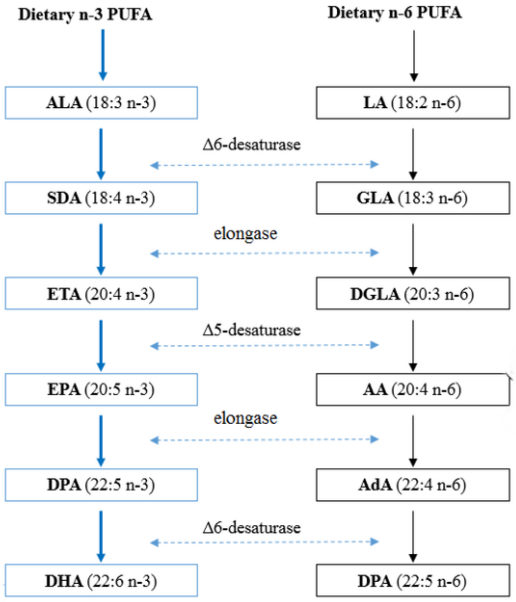


Figure 8 – Outline of the metabolism of the ω6 and ω3 essential fatty acids. Adapted from Jiajie *et al.,* 2014.

1.3.3. Lipid classes in fishery products

In fishery products, the lipids are composed mostly by Triacylglycerol (TAG) and phospholipids (PL), which vary in inverse proportion according to the fat content (Khan et al., 2011). However, cholesterol (CH), metabolic products and small amounts of these unusual lipids such as glycolipids, are also part of the lipids in the fish (Diana, 2009).

TAG is the form in which fat energy is stored in fish muscle, as reserve lipids. Each TAG consist in a glycerol molecule in which each hydroxyl group is esterified with one molecule of a free fatty acid. Most lipids are ingested in the form of TAG but must be degraded to fatty acids for absorption. The phospholipids are structural lipids, and composed of two apolar fatty acid molecules attached to a glycerol molecule, a phosphate group and a polar molecule. Phospholipids are not triglycerides. The storage and use of lipids are generally associated with seasonal changes in abundance of food and the fish metabolic requirements.

1.3.4. Fatty fish *versus* lean fish

According with the lipid content, the fish can be generally classified like lean when the content lipid in the edible is less than 5%. Hake, crustaceans, cod and sea bass are examples of lean species. Fish are considered semi-fatty when the lipid percentage is between 5% and 10% e.g. trout, bass and gold. Fatty fish have a fat percentage greater than 10%, at least once in a year. Fat species include pelagic fish like Atlantic salmon, sardines, mackerel, and herring (Huss *et al.,* 2004).

Most of aquaculture fish have a very rich diet in lipids to maximize growth rate, which leads to their stall meat to have a higher fat content than the wild species. Existing lipid content in the fish can vary over a wide range (Kolakowska *et al.,* 2003). However, the existing fat content in fish tends to vary not only between fish species, but also season, maturity and type of food available.

1.3.5. Importance of fish lipids in human nutrition

Lipids are composed of compounds responsible for the flavor, aroma and texture of a food, thus facilitating the acceptance by the consumer Interest in the fatty acids in the diet, especially the w3 family, has grown steadily since the 70s of the last century. It was observed that the populations of Eskimos in Greenland had a reduced incidence of cardiovascular disease but practiced a diet lipid (Dyerberg et al., 1975). Currently fatty acids, especially those of the ω3 family, are almost universally recognized as having beneficial effects on a variety of human diseases, such as cardiovascular diseases, inflammatory diseases, and neurological diseases (Tocher, 2009).

The consumption of fishery and aquaculture products contributed to numerous nutritional benefits that contradict the diets of most industrialized countries (Oehlenschläger, 1997). Indeed, fishes are an important source of nutrients, in addition to the ω3 fatty acids to a healthy diet. With few exceptions, most of the fish is low in saturated fats, cholesterol and carbohydrates, and high in protein, micronutrients, vitamins, and minerals and especially rich in fatty acids of ω3 family (Afonso, 2009). Thus, even when ingested in small quantities, fish plays an important nutritional effect by providing essential amino acids, essential fatty acids and micronutrients that are not found in diets which are based on vegetable products. The Commissions of Health and Welfare recommend consumption of diets with higher ω3 fatty acid content and a balanced ω3 / ω6 ratio, so it is urgent to encourage fish consumption among the population, especially seafood due to its richness in ω3 fatty acids.

2. OBJECTIVES

This study intends to investigate how the total lipid content and profile of fatty acids are influenced by the time of capture of the meagre produced in aquaculture.

3. MATERIALS AND METHODS

3.1. Samples and biometric parameters

This experimental work focused on meagre produced in April and November. These samples were collected from an established creation in the earthen ponds in the IPMA research center based in Olhão, in the region of Algarve in the south of Portugal, and then brought to IPMA in Lisbon to be studied in the laboratory. A total of nine samples were collected: three in April and six in November. The fish was slaughtered in the research center and biometric parameters were measured (weight and length). Muscle samples to be analyzed were prepared, freeze-dried and packaged in Eppendorf's in the fish production research center. Figure 9 shows an example of a sample. In the analytical facilities of IPMA the different samples were grounded in a mortar and weighted.

Figure 9 – Samples of the meagre.

3.2. Determination of the Chemical Composition

The chemical composition of the muscle of the meagre was determined on laboratory tests in order to detect possible differences that may exist between the seasons of April and November.

3.2.1. Ash

The determination of ash was carried out according to the standard AOAC (1995). The crucible was weighed, previously dried in an oven with temperature at 150ºC for one hour. Thus, it is ensured that any moisture present in the capsule is eliminated. Soon after the crucible was placed in the desiccator, and with the help of tweezers the crucible was removed and weighed on the analytical balance. The crucible weight was recorded. 5 g of sample were placed in the crucible, and then placed in an oven at 105ºC for one night.

Table 5 - Material and reagents used in determination of the chemical composition.

Materials
Crucible
Tweezers
Desiccator
Analytical Balance
Greenhouse
Muffle
Laboratory materials

After this time the crucible was removed from the oven, with the aid of tweezers, and cooled in the desiccator. Then the sample was placed again in the oven at 500°C-550°C, with destruction of the organic matter, without appreciable decomposition of the constituents of mineral residue or loss by evaporation. Incineration should be made until the ashes turn white or slightly gray.

Figure 10 – The determination of the ash.

After the crucibles reached the indicated temperature, they stayed one night in the oven. Afterwards the crucibles of the muffle were taken out with tweezers, carefully, for the ash not to disperse. They were put in the desiccator to cool for 40 minutes. After this time, the first weighing of the crucibles with ash was done. The crucibles were put again in the oven at 550ºC for 30 minutes. Afterwards, the crucibles were withdrawn into the desiccator to cool. After cooling the weighing of the crucibles with ash was done again. In the second weighing the ash weight increase means that the ash is ready and doesn't need to return to the oven. The ashes were calculated as follow:

$$\%Ash = \frac{Mass_{crucible\ ash} - Mass_{crucible}}{Mass_{crucible\ sample} - Mass_{crucible}} \times 100 \qquad \text{(Eq. 1)}$$

3.2.2. Total Lipids

The Lipid content was determined by the Soxhlet method. This method of quantification of lipid is based on the extraction of the lipid from a wet sample, by carrying out several rinses with ethyl ether under reflux.

In determining lipid content by the Soxhlet method 5 g of the sample were weighed, placed into an identified capsule, and added two tablespoons of anhydrous sodium sulfate homogeneously. Afterwards this mixture was transferred to the filter paper Wht n1, dragging all sample remains for the filter paper and folding the paper to form a cartridge which is then placed inside the extractor. Afterwards diethyl ether was added to the flask previously weighed and the extractor was placed with the sampler cartridge.

Ether was added in an amount sufficient to a Soxhlet and a half, in the adaptation to a cooler balls. The hot plates were kept under heating, with the continuous extraction for 8 hours (four to five drops *per* second) or 16 hours (two to three drops *per* second). Removed the bound paper filter, and distilled the ether transfer, the flask to the residue was extracted into an oven

at 105°C, for about an hour. The desiccator was used to cool to room temperature. Afterwards the weighing was done and all operations were repeated: heating for 30 minutes in the oven and cooling to a constant weight.

$$\% \, Lipid = \frac{Mass_{ballon\ lipid} - Mass_{ballon}}{Mass_{sample}} \times 100 \qquad \text{(Eq. 2)}$$

3.3. Extraction of Total Lipids

It was used the fat extraction method in fishery products and aquaculture for the extraction of total lipids in the meagre muscle, described by Bligh & Dyer (1959).

Table 6 - Material and reagents used in extraction of total lipids by the method of Bligh & Dyer

Materials
Polytron Ultra Turrax
Vortex (Heidolph, REAX 2000)
Centrifuge (Sigma, 2K 15)
Analytical balances, precision of ±0.001 g
Rotary Evaporator (Buchi 461 water bath)
Tubes with capacity of 20 ml and 18 ml.
Pear Balloons
Laboratory materials
Reagents
Methanol: Chloroform Solution (2:1)
Saturated solution of Sodium chloride, 1ml
Chloroform, 2 ml
Ultra-pure water, 2 ml

The first step in the extraction process is to weigh 1 gram of lyophilized sample (homogenized sample) in an analytical scale and to place it in the 20ml tubes that were already weighed and determined.

For the second step 5ml solutions of Methanol: Chloroform (2:1) was added to the tubes, in order to help the extraction of fats, shaking slightly in the polytron. After shaking, more 1ml of sodium chloride saturated solution was added to help the precipitation of proteins, shaking 30 seconds more in the polytron.

After this shaking more 2ml of chloroform was added, continuing to shake for 30 seconds and at last, more 2ml of very pure water were added. The tubes continued to be shaken for 1 minute, and then they were centrifuged in a centrifuge in 4ºC in 5000rpm for 10 minutes. In this way, the separation of phases occurred (watery phase and organic phase), and the watery phase (superior part) was collected with a Pasteur pipette and eliminated it. Again with another Pasteur pipette the organic phase was collected into other 18ml tubes previously filled up with anhydrous sodium sulfate, in order to eliminate the remaining water. These tubes were shaken slightly in a vortex and were put, again, in a centrifuge with 4ºC and 5000rpm. The organic phase was collected with a Pasteur pipette and was added into a 50ml pear balloon, filtering through a cotton column with anhydrous sodium sulfate. 2ml of chloroform were added to the anhydrous sodium sulfate tubes and shaken in a vortex. Once again, they were centrifuged as explained before. It was extracted, with a Pasteur pipette, the extract to the pear balloons that already had the extract from the first extraction. 3ml of chloroform were put to drag some eventual residuum of the cotton column with anhydrous sodium sulfate. The pear balloons were put in the rotavapor, to evaporate the chloroform in a bath with a temperature of 45ºC and 120rpm.

Finally, the weighing of the pear balloons was done, with a simple subtraction, to determine the amount of fat present in the sample. With the weighing done the determination of the amount of fat in the samples was done with the equation 1.

$$Amount\_fat = \frac{Pear\ mass_{final\ fat} - Pear\ mass}{Sample\ mass} \times 100 \quad (\%) \qquad \text{(Eq. 3)}$$

3.4. Determination of lipid classes

After the extraction of total lipids it is intended to evaluate which lipid classes are present in those fractions, though the TLC. This technique consists on the identification of the components present in the sample though the pattern comparison.

Before the injection of the sample into the silica plaques it was necessary to prepare the material to use, like the solution of elution of the samples.

Table 7 - Material and reagents used in determination of lipid classes with TLC.

Materials
Silica Gel Plates with 0,25mm thickness 20x20cm
TLC support with metallic pencil and ruler
Desiccator
Greenhouse
Elution chamber
Micropipette of total exclusion
Automatic Sprayer
Lab Materials
Reagents
n-Hexane
Diethyl ether
Formic acid
Phosphomolybdic acid hydrated 10% in Ethanol
The TLC standard mixture (TAG, CH, FFA, PC)

Firstly, the plaque was put into the greenhouse at 110ºC for 1 hour to active it from the silica, and then was left to cool in the desiccator in room temperature.

In the case of eluting solution, this consists of n-hexane:diethyl ether:formic acid, in the concentration of (65:35:1), leaving it in the inside of the chamber to promote the saturation for approximately 30 minutes.

The plaque was placed in the support and a line was drawn in the superior limit (which prevents the samples from eluting into the outside of the plaque) and the identification of the samples and the mixed pattern was done. Then, with the help of the ruler, 10ul of the samples were applied leaving a gap between the samples, as the following scheme in Figure 11 shows:

Figure 11 – The application of the TLC analytical plaque.

The plaque was placed into the elution chamber, leaving the solvent to elute until the superior line of the plaque. When the elution was completed the plaque was removed from the chamber, left to evaporate, and with the help of the automatic sprayer the plaque was pulverized with the Phosphomolybdic acid solution with 10% of ethanol. Then, the plaque was placed in the greenhouse at 120ºC where it stayed for approximately 1 hour for the revelation of the components of the respective lipid fractions, and to contribute for the darkening of the colour.

When revealed, the identification of the relative percentage of the different components was digitalized though the Quantity One program of PDI.

3.5. Determination of the fatty acids profile

The fatty acids profile is determined though the method described by Lepage and Roy (1986), modified by Cohen *et al.,* (1988) by gas chromatography. This method consists on the transesterification of the fat acids into methyl esters (FAME – Fatty Acid Methyl Esters). The most common methods for determining the fatty acid composition of animal fats is gas chromatography (GC). For that purpose, the fats are usually converted to the corresponding methyl esters.

Table 8 - Material and reagents used in determination of the fatty acids profile.

Materials
Vortex (Heidolph, REAX 2000)
Centrifuge (Sigma, 2K 15)
Analytical balances, precision of ±0.001 g
Thermostatic Bath
Lab materials
Gas phase chromatograph equipped with an auto sampler and a call ionization detector.
Vortex (Heidolph, REAX 2000)
Centrifuge (Sigma, 2K 15)
Reagents
Ultra-pure water
Anhydrous sodium sulfate 99,9 %
n-heptano >99,3 %
Methanol 99,8 %
Acetyl chloride > 98 %

The samples were weighted on the analytical balance with the samples properly prepared (milled), on 15 ml tubes to approximately 300 mg. After this the acetyl chloride:methanol (1:19, v/v) solution was prepared. This solution was prepared in the fume hood and the glass in a cuvette with ice. The function of these two compounds promotes an exothermic reaction, where heat is released. Firstly the methanol is added in the beaker with a Pasteur pipette, dropwise along the beaker walls of acetyl chloride. 5 ml of methanol solution of acetyl chloride were added to each tube containing the sample. The tubes were stirred in vortex for 30 seconds and added 50 microliters internal standard (21:0). After placing in a water bath at 80 C for one hour they were removed and left to cool.

To each tube 1 mL of Milli Q water and 2 ml of n-heptane were added and the tubes vortexed were stirred again and centrifuged at 3000G for 5 minutes.

The organic phase (top) was collected, where the methyl esters for a vial are found, by filtering through a cotton column with anhydrous sodium sulfate so as to ensure that no fraction of the aqueous phase was transferred to the vial.

Then, all the samples needed to be concentrated were placed under a continuous flow of nitrogen, in order to promote their evaporation, followed by addition of the desired volume of n-heptane.

Afterwards, the analysis of the methyl esters of fatty acids was done, by injecting 2 microliters in a gas phase chromatograph, Varian CP-3800 with flame ionization detector (FID). The separation is effected in a fused capillary column DB-wax polyethylene glycol (30m x 0.25 mm id x 0.25 µm) using helium as a carrier gas. The method of analysis lasted for 40 minutes per injection, and during this time, the column was heated to 180ºC, increasing about 4ºC for 25 minutes, until it reached 220ºC.

At the end of the reading one obtains a chromatogram which has to be analyzed. The fatty acids present in the sample are identified by comparing the retention time obtained for each of them and the obtained time in the same existing fatty acids in the pattern of Sigma-Aldrich. Finally, the step of quantification of the different fatty acids as a function of its peak area followed: the peak area of internal standard (21:0), the mass of the sample weighed, and the total area of the fatty acids from the sample.
To calculate the relative percentage of total fatty acids excluding the area of the Internal Monument, the equation 2 was used:

$$\%\text{Relative} = \frac{\text{Area}_{\text{fat acid}}}{\text{TotalArea}_{\text{Chromatogram}} - \text{Area}_{\text{PI(21:0)}}} \times 100 \qquad \text{(Eq. 4)}$$

After the conversion of the area in percentage relative the calculation of the mean and respective standard deviation was done, in order to draw graphs.

3.6. Statistical analysis

All data are presented as mean ± standard deviation. Data from the studies were subjected to analysis of variance (one-way or factorial ANOVA) using Excel and the Statistica TM v.8 Software (StatSoft Inc., 2007). Statistically significant differences ($p<0.05$) between samples were determined according to Unequal N HSD test.

Hierarchical cluster and principal component analysis (PCA) were run using StatisticaTM v.8 Software (StatSoft Inc., 2007). The clustering process involved three steps: data standardization; assessment of a dissimilarity measure among samples; and the use of a grouping technique. In the present study, data standardization was done in order to provide null means and variance equal to one for each sample. The Euclidean distance was used as a dissimilarity distance. Ward's method was used as the grouping technique (Ward, 1963), which involves an agglomerative clustering algorithm starting out at the leaves (n clusters of size 1) and continues to the trunk until all the observations are included into one cluster which is referenced as most appropriate for quantitative variables.

Principal component analysis, a multivariate exploratory technique, was also run using StatisticaTM v.8 Software. All variables were mean centered and standardized (scaled) to unit variance prior to analysis, i.e. correlation matrix. The principal components were obtained by computing the eigenvalues and eigenvectors of the studies data correlation matrix. For each component of the PCA, a score for each sample was calculated as a linear combination for each quality variable and the contribution of each variable to the PCA score was deduced from the parameters loading for the factor. A bi-dimensional representation of this multidimensional set was made for the principal components that accumulated a significant percentage of original information, above 70%, which is considered sufficient to define a good model for qualitative purposes.

4. RESULTS AND DISCUSSION

4.1. Biometric parameters

Table 9 shows biometrics of individuals belonging to both season group analyzed. Thus, it can be stated that the average weight obtained for the 6 animals analyzed in November was 4797.2 g with emphasis on the highest weight in November (6171g) and the lowest in April (3315g).

On the other hand, in relation to total length, animals in April (68.5cm) are smaller than in November (71.9cm). In general, the length of meagre captured in November ranges between 71.9 cm and 86.0cm, with a mean value of 80.0 cm.

Table 9 – Biometric parameters of meagre produced in April and November: Total weight (TW) Total Length (TL).

April			November		
TW (g)	**TL(cm)**	**Gender**	**TW (g)**	**TL (cm)**	**Gender**
3636	74.0	M	6171	86.0	F
3315	68.5	F	5558	83.9	F
3882	78.4	F	5003	80.6	F
			4586	81.5	F
			3989	76.2	F
			3476	71.9	F
Mean ± SD			**Mean ± SD**		
3611 ± 284.3	73.6 ± 5.0		4797.2 ± 995.2	80.0 ± 5.2	

4.2. Ash and Lipid Contents

Table 10 – Ash and lipid contents of muscle of meagre by dry weight.

	Ash (%)	Fat (%)	EPA+DHA (%)
April	5.06	3.32	12.94
November	4.52	8.64	10.98

Table 10 presents the results obtained in a dry basis for the overall fat and ash composition of the muscle of meagre slaughtered in the months of April and November. At Table 10 it appears that the ash and lipid content varies greatly over time in meagre produced in aquaculture. This variation is statistically significant ($p<0.05$).

The fat content, including the content of EPA+DHA, is one of the fundamental aspects in the present study. It was analyzed how the consumption of meagre contributes to the polyunsaturated fatty acids needs of the human body.

4.3. Characterization of lipid composition of feed

The current trend in fish feed production is to increase lipid content, improve feed conversion, decrease the amount of waste produced by the fish and special attention is being given to the development of feeds that maximize nutrient retention and minimize nutrients loss. The fatty acid profile of meagre fed is showed in Table 11. Fish fed granulated by 5mm had a high content of MUFA (35.6±3.1 mg/g), in which stand the oleic (18:1(ω9)-25.31±2.28 mg/g) and palmitoleic+hexadecenoic (16:1(ω9)+(ω7)-6.15±0.54 mg/g) acids. The quantity of SFA was higher than MUFA, due to high content in palmitic (16:0-23.46±2.08 mg/g) and stearic (18:0-5.54±0.43 mg/g) acids. The feed was found to have a reasonable content of PUFA (31.4 ± 2.4 mg/g), with a special level of LA associated with a vegetable origin, is also important to stress the fatty acids EPA and DHA.

Figure 12 – Ration of meagre bred in November.

Table 11 – Fatty acid methyl ester (FAME) profile of meagre fed granulated in 5mm. Values are in mg/100g of total muscle fatty acids, n.d.-not detected.

Fatty acid (mg/100g)	Mean	SD
11:0	0.07	0.01
14:0	2.94	0.27
15:0 iso	0.11	0.01
15:0 ante-iso	0.08	0.03
15:0	0.39	0.04
16:0 ante-iso	0.04	n.d.
16:0	23.46	2.08
17:0 iso	0.22	0.02
17:0	0.43	0.03
18:0	5.54	0.43
19:0 iso	0.12	0.01
19:0	0.24	0.02
20:0	0.27	0.02
22:0	0.09	0.01
Total SFA	**34.00**	**2.99**
16:1(ω9)+(ω7)	6.15	0.54
17:0	0.09	0.02
18:1(ω9)	25.31	2.28
18:1(ω7)	2.26	0.16
18:1(ω5)	0.06	0.01
20:1(ω9)	1.01	0.07
20:1(ω7)	0.07	0.01
22:1(ω11)	0.55	0.03
22:1(ω9)	0.14	0.01
Total MUFA	**35.64**	**3.12**
16:2(ω4)	0.45	0.03
16:3(ω4)	0.51	0.06
16:3(ω3)	0.09	n.d.
16:4(ω3)	0.42	0.03
18:2(ω6)	15.59	1.30
18:3(ω6)	0.10	0.03
18:3(ω4)	0.09	0.01
18:3(ω3)	1.36	0.11
18:4(ω3)	0.77	0.06
20:2(ω6)	0.17	0.01
20:4 (ω6)	1.01	0.06
20:4 (ω3)	0.25	0.02
20:5(ω3) EPA	4.02	0.35
21:5(ω3)	0.16	0.02
22:5(ω6)	0.25	0.02
22:5(ω3)	0.50	0.04
22:6(ω3) DHA	5.69	0.30
Total PUFA	**31.43**	**2.44**
Total (ω3)	**13.25**	**0.93**
Total (ω6)	**17.13**	**1.42**

Table 12 – Class of lipids present on the meagre fed granulated in 5mm (%-TL:total lipid classes).

(%)	TAG	FFA	CH	PL	TL
Granulated 5 mm	66.0	17.4	13.2	2.7	18.2

Looking at the meagre fed it is found that, by the frame, in terms of total lipids (TL), it contained about 18.2% of dry weight. In terms of lipid classes, it is noted that the predominant class is the TAG, followed by FFA.

4.4. Impact of seasons on composition of total lipid content in muscle of meagre

Meagre varies its chemical composition seasonally. The lipid fraction is the component showing the greatest variation. In this study, in the muscle of meagre that was bred in aquaculture in two different seasons (Spring and Autumn), the amount of total lipid content was analyzed and it was observed that the values of lipid profile were significantly higher in Autumn.

During the spring period the meagre feeding behavior can be affected by low water temperature. In contrast, in November the meagre has its maximum feed intake stage, because it lived a long period in warm waters. Warm months may influence also the size and reproductive potential of the fish.

Linoleic acid (LA) is one of the most important acids of feed and, in the warmer months as the water temperature increases, meagre tends to consume more feed. It is therefore expected that the LA content in the muscle should increase.

As it was stated above, the highest lipid content was observed in Autumn. It may be noted that the change of season has a significant impact on the lipid metabolism and deposition in fish.

Significant seasonal differences in the lipid content and fatty acid composition were found in this study ($p<0.05$). The lipid content varied significantly, from 3.15% dry weight (dw) in April to 8.64% (dw) in November. The SFA accounted for 30.50% FAME in April, while MUFA contributed 34.70% FAME in November. Contrarily, PUFA made up to a minimum of 28.69% FAME in November and a maximum of 39.80% FAME in April.

Table 13 – Mean weight percentage of the main fatty acids in the muscle of meagre produced in two seasons. (mean±sd, n.d.-not detected).

Fatty acid (%)	Spring mean	Autumn mean
11:0	0.01±0.01	n.d.
14:0	2.13±0.27	2.86±0.12
15:0 iso	0.08±0.01	0.04±nd
15:0 ante-iso	n.d.	0.09±nd
15:0	0.36±0.04	0.44±0.02
16:0 ante-iso	0.72±0.02	0.26±0.01
16:0	18.71±1.41	23.98±0.67
16:1ω9+ω7	3.33±0.31	5.88±0.22
17:0 iso	1.18±0.02	0.20±0.02
16:2ω4	0.50±0.03	0.47±0.04
17:0	0.41±0.02	0.47±0.01
16:3ω4	0.33±0.03	0.48±0.01
17:1	0.13±0.02	0.14±0.05
16:3ω3	0.67±0.05	0.34±0.04
16:4ω3	0.39±0.06	0.31±0.01
18:0	7.46±0.16	6.12±0.04
18:1ω9	20.11±0.40	23.64±0.23
18:1ω7	2.37±0.04	2.18±0.03
18:1ω5	0.09±0.01	0.08±nd
19:0 iso	n.d.	n.d.
18:2ω6	19.23±0.25	11.54±0.12
18:3ω6	0.03±0.04	0.10±nd
19:0	0.19±0.01	0.21±nd
18:3ω4	0.17±0.03	0.17±0.03
18:3ω3 LNA	1.57±0.04	0.87±0.01
18:4ω3	n.d.	0.57±0.02
20:0	0.24±0.02	0.24±0.01
20:1ω9	1.80±0.15	1.52±0.04
20:1ω7	0.06±0.02	0.06±0.01
20:2ω6	0.30±0.03	0.21±0.02
20:4ω6 AA	1.47±0.11	1.12±0.03
20:3ω3	0.04±0.03	0.02±0.01
20:4ω3	0.24±0.03	0.27±0.01
20:5ω3 EPA	2.58±0.20	3.43±0.10
22:0	0.04±0.06	0.09±0.01
22:1ω11	0.97±0.12	0.87±0.04
22:1ω9	0.25±0.02	0.20±0.02
21:5ω3	0.10±0.14	0.15±0.01
22:4ω6	0.03±0.04	0.06±0.02
22:5ω6	0.57±0.08	0.39±0.04
22:5ω3	0.91±0.13	0.78±0.06
22:6ω3 DHA	10.36±1.53	7.98±0.49

4.5. Total lipids and lipid class

This analysis aims to determine simultaneously the dominant classes in the meagre muscle and verifying the existence of seasonal variations. The measurement of total lipids content and its deposition was made through the analysis of the muscle. Figure 13 shows the lipid content of the muscles in the two investigated seasons, spring and autumn.

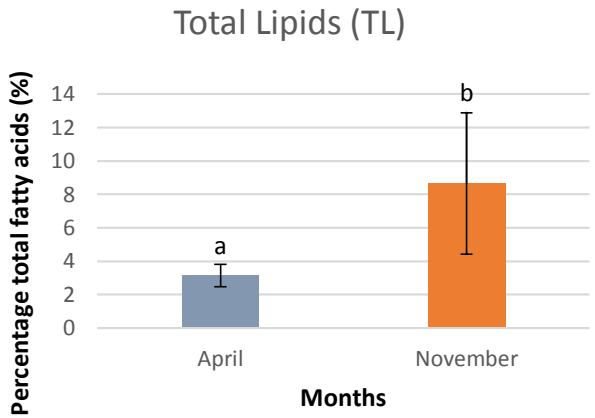


Figure 13 – Total content of lipids percentage in April and November (a and b are the variance of the TL content, and are significantly different ($p<0.05$)).

Analyzing total lipids (TL) it is observed that there is a certain percentage difference between both seasons. In April the lipid content, as expected, contained about 3.2% and in November increased to 8.7% in dry weight. Total lipid content in Spring is significantly ($p<0.05$) lower than in Autumn.

Fatty acids were found in the fish preferably in the form of triacylglycerol (TAG) and phospholipids (PL). Only a very small fraction is lying in the free fatty acids (FFA), which are often associated with the occurrence of adverse reactions of hydrolysis. Sometimes they may also be found, but to a lesser extent, in the form of cholesterol (CH).

Table 14 – Mean values of lipid classes in the muscle. Values are (mean ± standard deviation), Analyzed by means of ANOVA using two samples (A , Not Significant).

	TAG+FFA(%)	CH(%)	PL(%)	ANOVA
April	84.6±3.5	10.0±2.6	5.4±0.8	A
November	83.2±4.7	12.1±3.5	4.7±1.8	A

Looking at the dominant lipid classes in the samples under this study it is seen that, as expected, the initial portion is the class of the triacylglycerol +free fatty acids (TAG+FFA) which is more significant. The sum of (TAG+FFA) was used to standardize the obtained levels.

Analytical TLC showed that the muscle consists in April for TAG+FFA (84.6%), CH (10.0%) and PL (5.4%) (Table 14). In November the fish constitution was different, and TAG+FFA content decreased (83.2%), CH content increased (12.1%) and PL content decreased (47.2%).

4.6. Fatty acid profiles

Once it is known which lipids classes exist in the initial portion of meagre, the next important nutrients to investigate are its fatty acid composition. The fatty acid profile of the fish is largely determined by its nutritional value. The fatty acid profile can be seen in the graphs below for each fatty acid. Each figure describes the variation of the average fatty acids in percentage. At the same time it is represented the respective standard deviation by vertical bars.

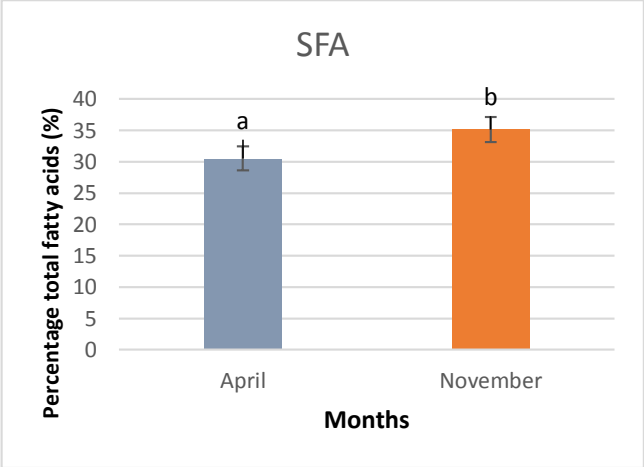


Figure 14 – Total SFA content in April and November (a and b are the variance of the SFA content, and are significantly different ($p<0.05$)).

In the first figure the mean percentages of SFA of the meagre muscle samples are represented in the months of April and November. The amount of SFA raised with post-summer period in the month of November is indicated above. For the muscles of both seasons under this study, myristic acid (C14:0) was the most abundant followed by palmitic acid (C16:0). These maximus appeared in November. The palmitic fatty acid is a potential source of metabolic energy.

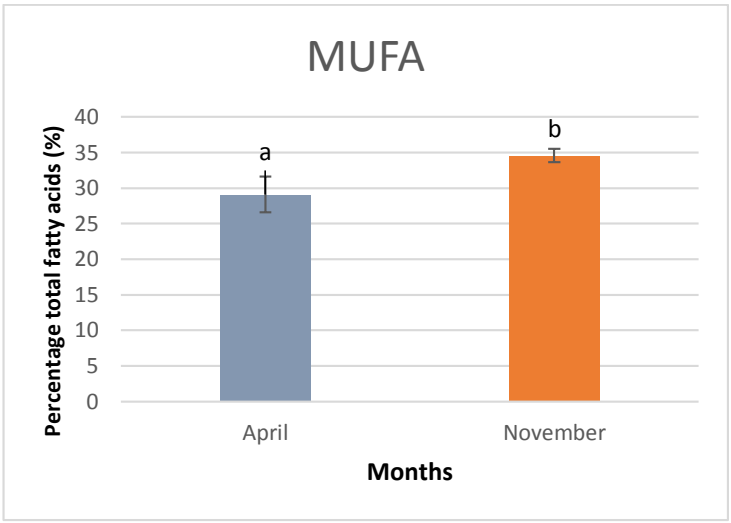


Figure 15 – Total MUFA content in April and November (a and b are the variance of the MUFA content, and are significantly different ($p<0.05$)).

In respect to MUFA of total muscle lipids, significant differences were found between the two seasons ($p<0.05$), and in November the total MUFA had the highest content (34.70%). In both seasons the predominant acid was 18:1(ω9+ω7+ω5), followed by 16:1(ω9+ω7).

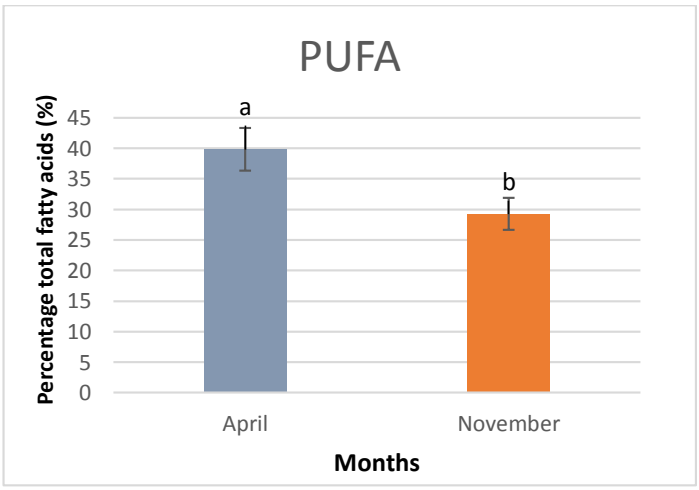


Figure 16 – Total PUFA content in April and November (a and b are the variance of the PUFA content, and are significantly different ($p<0.05$)).

With regard to PUFA of the total muscle lipids there were significant differences ($p<0.05$) between the two seasons under study, concretely the total levels of PUFAs. In the season of April PUFA had a higher value (39.80%) than in the season of November (28.69%), as already mentioned above (Figure 16).

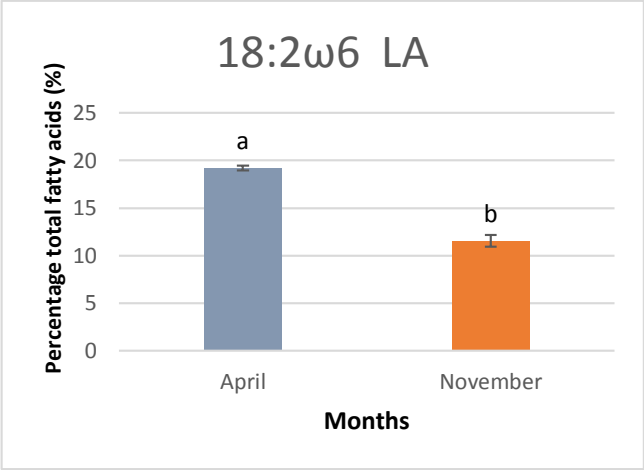


Figure 17 – Total LA content in the muscle in April and November (a and b are the variance of the LA content, and are significantly different ($p<0.05$)).

The group of PUFA was mostly represented by linoleic acid (LA, 18:2ω6). Regarding ω6 family, the most abundant was LA, comprising 19.22 % of PUFAs as a percentage of total fatty acids in LA. As to LA, the figure 17 shows that the muscle in April is rich in this fatty acid percentage of total fatty acids.

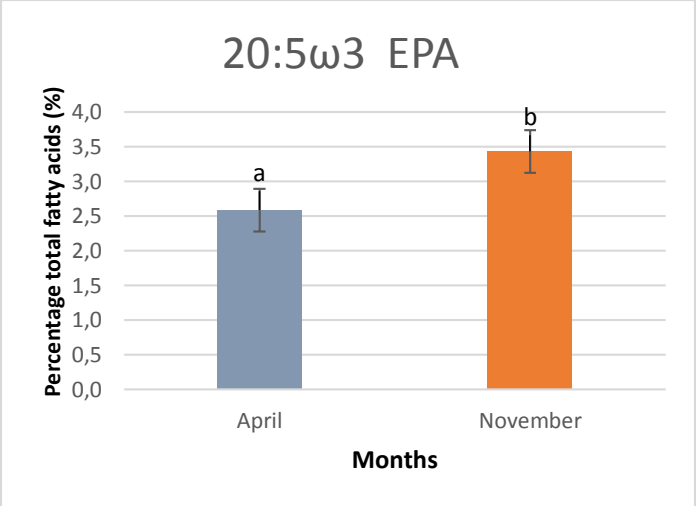


Figure 18 – Total EPA content in the muscle in April and November (a and b are the variance of the EPA content, and are significantly different ($p<0.05$)).

Differences in the levels of EPA and DHA were observed also in the class of PUFA. The DHA and EPA are the dominant ones. The EPA content varies widely between seasons, having recorded its highest value in November (3.50% and 2.9 g/100g/dry wet).

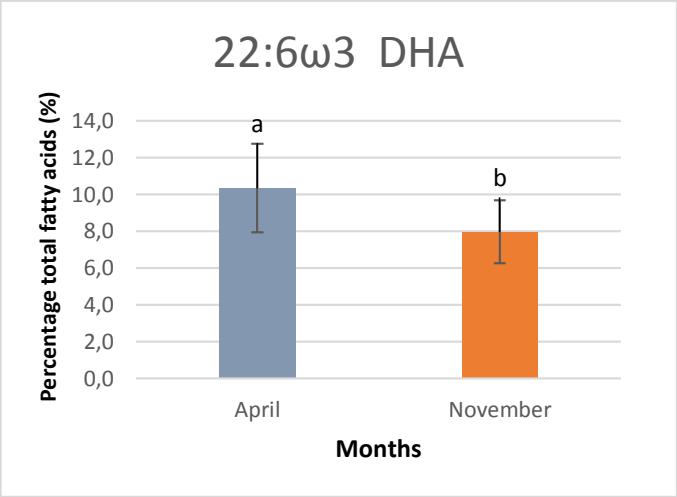


Figure 19 – Total DHA content in the muscle in April and November (a and b are the variance of the DHA content, and are significantly different ($p<0.05$)).

In both seasons the meagre samples had a good proportion of DHA. The meagre in November was the one which had the lowest percentage of DHA (7.59%) while the meagre in April registered the highest level as a percentage (10.35%) and in absolute value (6.6 g/100g/dry wet).

The DHA content in the muscle presents some variation, which increases from November to April (Figure 19). The presence of higher amounts of DHA in the muscle compared with the remaining fatty acids studied support a higher proportion of a structural fraction associated with a lower lipid content seems to be influenced by the phospholipids.

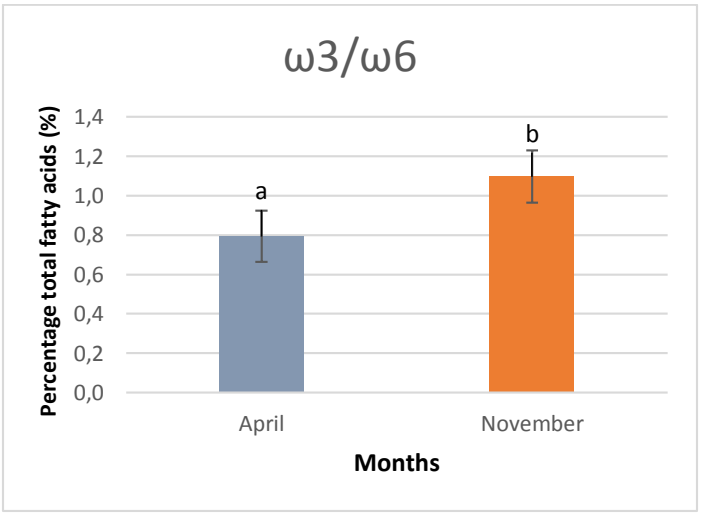


Figure 20 – Total ω3/ω6 in the muscle in April and November (a and b are the variance of the ω3/ω6 content, and are significantly different ($p<0.05$)).

All results show that the total ω3/ω6 ratios vary accordingly to the season of meagre, being lower in Spring (0.79%) and higher (1.07%) in Autumn. Note that in this study the meagre in November showed much lower percentages of fatty acids ω6 and higher values for the ω3/ω6 ratio.

Looking at the lipid profile, the meagre in November showed that its body contains significantly higher amount of MUFA, followed by a SFA. Within the PUFA, although contains low levels (28.69%), the majority were found to be LA, EPA and DHA fatty acids, wherein the distance LA is to present contents in the order of 11.48%.

The concentration of the total SFA was significantly different in the seasons, peaking in the autumn. The MUFA content also showed significant differences ($p<0.05$) in different seasons, the highest in the warmer months, between spring and autumn. Finally, the PUFA content showed higher levels for lower water temperatures. It is concluded that the season has a major influence on the lipid composition of muscle meagre. November in this study was the month in which the water temperature was above however, the SFA, the MUFA and PUFA, statistical analysis demonstrated that there was significant variation in lipid content in different seasons.

The results showed the lowest EPA + DHA contents in autumn, showing season dependence for these acids. A ω3/ω6 ratio of 0.79% was observed in the muscle of meagre in spring. This ratio raised until 1.07% for meagre in autumn in aquaculture. The relative amount of these acids changed significantly between seasons of collect.

The composition distribution and the relationship among the ω3 and ω6 family in fish are mainly influenced by 3 factors: genetic (e.g. species, stage of development) environment (e.g. marine environment, water temperature, salinity) and diets (Ackman, 1982).

Environmental factors such as salinity and temperature influence the fish metabolism, affecting their requirements for fatty acids. Although the influence of environmental parameters in the determination of lipid requirements for fish is important, the food is the factor that most contributes to the fatty acid profile of triacylglycerol in animals (Henderson and Tocher, 1987).

4.7. Hierarchical cluster and principal component analyses

Hierarchical cluster and principal component analysis were used to establish a relationship between the time of capture of the fish and lipid composition. The data set included A1, A2, A5 (samples from April), N1, N2, N3, N4, N5, N6 (samples from November), and R5 (Ration of meagre bred in November). The quantitative variables corresponded to A1, A2, A5, N1, N2, N3, N4, N5, N6, R5. A transformation matrix was calculated to make a projection of the samples into a space described by eigenvectors that describe the direction of greater spread of these samples.

The cluster analysis (Figure 21) shows that the samples in question form two distinct groups to a Euclidean distance about 8, so it can be said that the studied samples are different. The Euclidean distance of 8 shows that the April samples form a separate group of November samples. The first group was formed by the muscle of meagre in November and meagre fed. The second group was formed by the muscle of meagre in April, with the fish grow up in November with lower weight 3476g (N6).

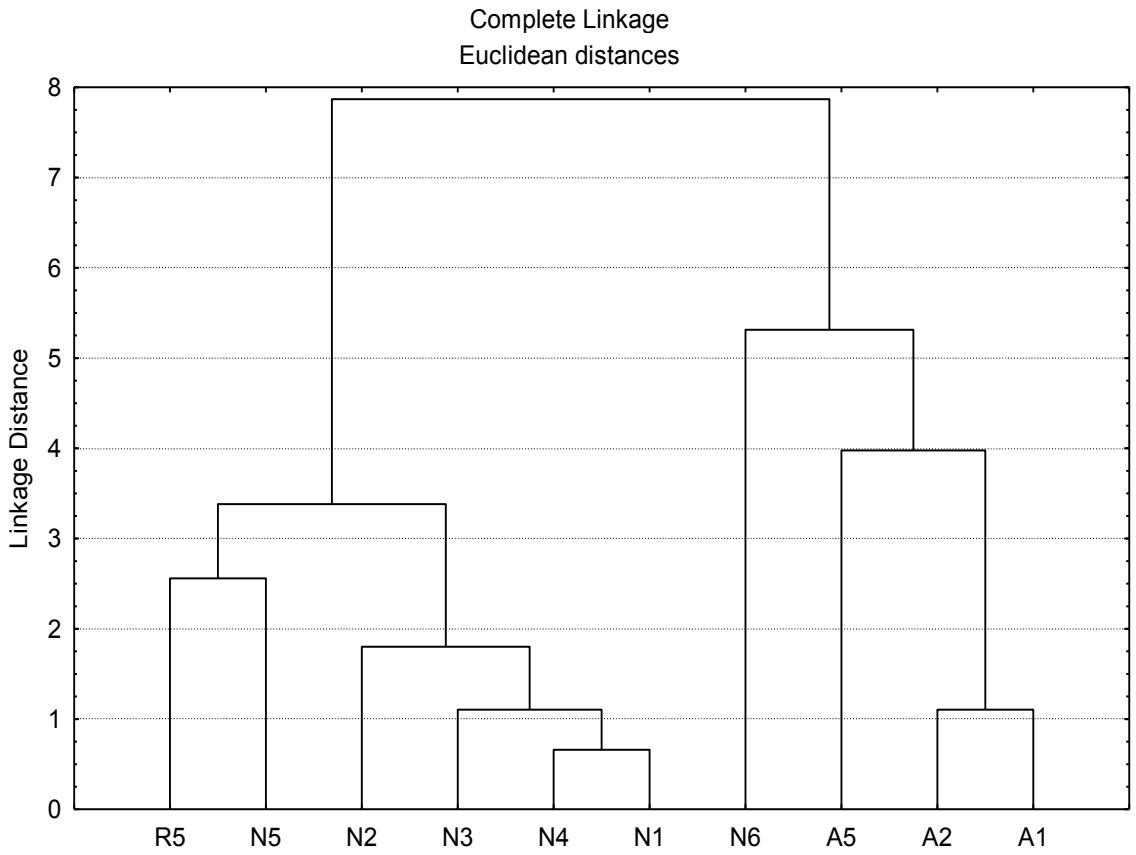


Figure 21 - Fish samples grouping as determined by cluster analysis.

Principal components analysis allowed to explain 94.43 % of the original data variability in the first two dimensions (principal components, PC, 1 and 2) which is considered adequate to define a good model for qualitative purposes since a significant percentage of original information (>70%) was accumulated within the first two PC's.

The PC1 is accounted for the highest proportion of the variation (68.64%) in the data and was most heavily loaded with SFA, MUFA, PUFA, 18:2 ω6, EPA, DHA, EPA+DHA, ω3 and ω6. The ω3/ω6 is described by PC2, accounting for 25.8% of data variability.

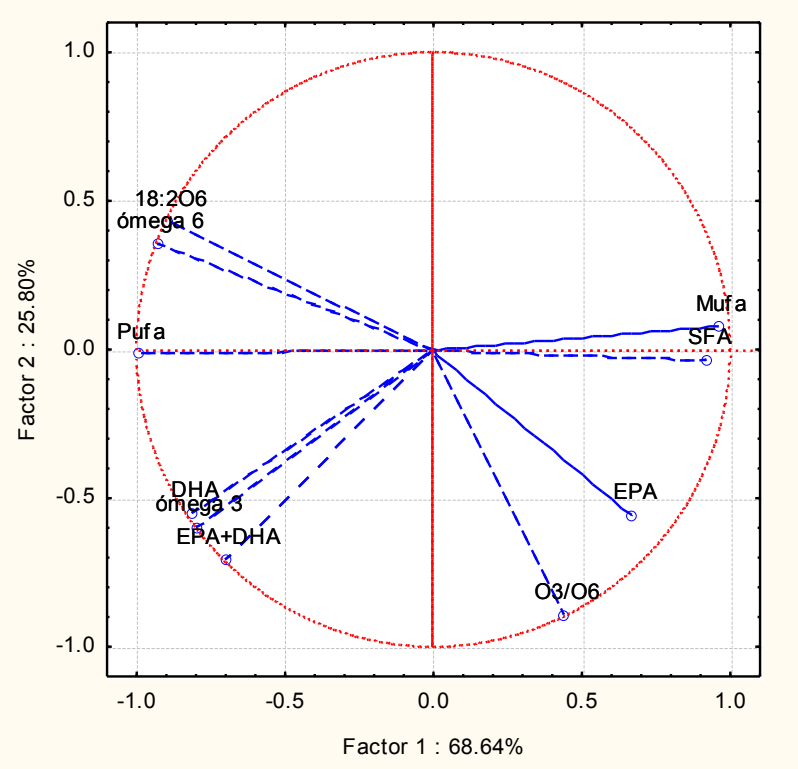


Figure 22 – Projection of the variables in the plane defined by the components 1 and 2.

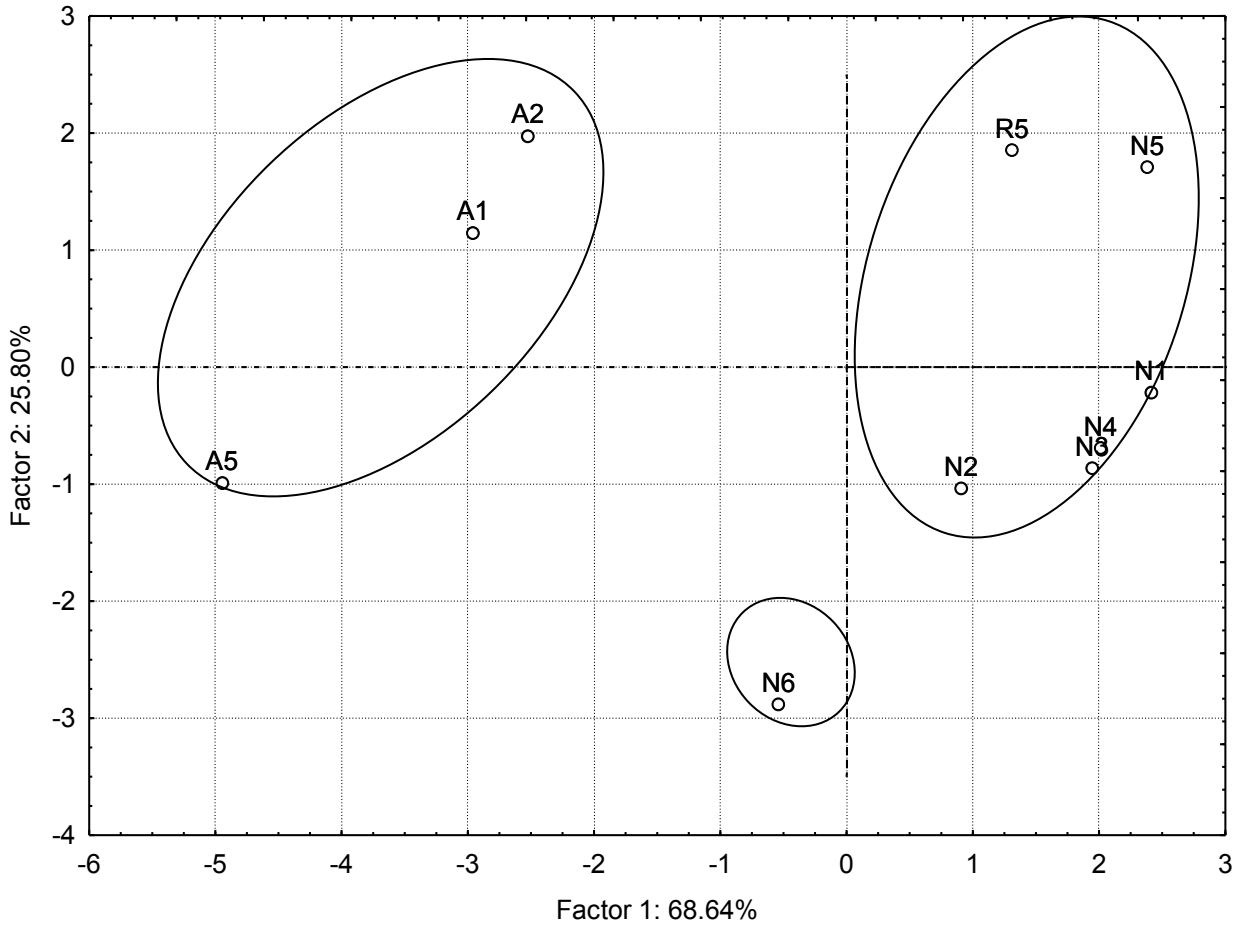


Figure 23 – Projection of the samples in the plan defined by the components 1 and 2.

The projection in the plane defined by the first two principal components in different seasons analyzed in April and in November is shown in Figure 23. Within the score plot, sample grouping as indicated by the hierarchical cluster analysis (Figure 21) is also shown.

The correlation between the amount of ration eaten and the observed fatty acids is lower in April, because the meagre ate small amounts of ration in this month compared with November.

5. CONCLUSION

With this study can be concluded that,

- The meagre is a low fat fish.
- The amount of total lipids varies with the season of capture, but is significantly higher in November.
- The meagre has high amounts of DHA compared with other fish. In April these values are even higher, because the fish is lean.
- Regarding the profile of the meagre muscle fatty acids, it was found that samples from April showed significantly higher content of omega3 fatty acids and omega6, especially LA and DHA.
- Fishes of different dimensions seem to present different content.
- It is concluded that the season of capture has a major influence on the lipid composition of meagre muscle, probably explained by the water temperature. November in this study was the month in which the water temperature was higher. However, a significant variation was found in lipid content in different seasons regarding the SFA, the MUFA and the PUFA.
- The results indicate that a larger ω3/ω6 ratio is observed in meagre in November, turning its consumption even more beneficial.

Even with some differences between seasons in the year the meagre can always be considered a balanced food that contributes for the consumer health and well-being. As a result aquaculture looks very promising in the meagre production.

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